Deciphering the cellular and molecular response of human dopaminergic neurons to mitochondrial stress

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Résumé

Parkinson's Disease (PD) is characterised by the degeneration of numerous neuronal cell types, with a predominant loss of dopaminergic (DA) neurons from the *substantia nigra pars compacta* responsible for the motor symptoms of the disease. Several lines of evidence emphasize the role of mitochondrial impairment in PD, such as defects in mitochondrial complex I activity and in mitochondrial DNA homeostasis in PD patients' brain tissue. Moreover, two autosomal recessive PD-linked genes, *PARK2* and *PINK1*, encode crucial proteins involved in mitochondrial quality control mechanisms. These proteins are ubiquitously expressed in neuronal and non-neuronal cells, just as mitochondrial alterations were observed in many cellular subtypes. Yet, these defects lead to the preferential degeneration of DA neurons. This raises the hypothesis that DA neurons specifically display high vulnerability to mitochondrial stress, resulting in irreversible mitochondrial alterations and DA neuronal death in PD. Although the PINK/PARKIN mitochondrial quality control system, as well as the ATF4-mediated Integrated Stress Response (ISR), have been identified as key mechanisms underlying the mitochondrial stress response, the complete and integrated sequence of cellular and molecular mechanisms involved in human DA neurons is still poorly understood. In this context, using LUnd Human MESencephalic (LUHMES)-derived DA neurons, we aimed at deciphering the mitochondrial stress response of human DA neurons, to further understand their specific vulnerability and its contribution to PD pathophysiology. We focused particularly on stress-associated changes in non-coding elements of the genome, as long non-coding RNAs (lncRNAs) and open regions of the chromatin regulate many developmental and cellular processes, implicating them in many human diseases. Their study is especially relevant in the context of pathologies linked to alterations of particular cellular subtypes, such as PD, as they constitute highly cell-specific molecular signatures.

Upon mitochondrial stress induced by Oligomycin A and Antimycin, we first observed a widespread increase in mitochondrial fission, a process preceding mitophagy. At a molecular level, RNA-sequencing confirmed the involvement of the ATF4-ISR pathway, and more specifically the PERK-mediated activation of the ATF4-ATF3-CHOP pro-apoptotic network. This result was reinforced by the enrichment of ATF4 binding motifs in open chromatin regions in stress conditions. Importantly, unlike previous studies, we also found other ISR signalling pathways, i.e. IRE1-XBP1 and NRF2, to be triggered in DA neurons following stress. In line with these results, several ISR downstream pathways were upregulated, such

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as metabolic pathways, including one-carbon metabolism, and Unfolded Protein Response chaperones.

In parallel, we established the DA neurons repertoires of lncRNAs expressed in control (802 transcripts) and stress (659 transcripts) conditions. Among these, we identified 272 control-specific and 129 stress-specific lncRNAs, reflecting high cell-specificity. Gene ontology analysis on the lncRNAs' most probable targets, i.e. the adjacent or overlapped protein-coding genes, suggested a major contribution of stress-associated lncRNAs to the process of negative regulation of translation. This stress-triggered mechanism acts by modulating the formation of translation initiation complex EIF4F, thereby repressing protein synthesis. Thus, among the stress-associated lncRNAs, we observed a significant enrichment of transcripts nearby genes encoding either proteins that directly inhibit EIF4F assembly, or amino acid transporters that also participate to EIF4F regulation *via* the mTOR central nutrient sensor pathway.
Altogether, our results demonstrate for the first time the concomitant activation of several ISR signalling pathways in human DA neurons in response to mitochondrial stress. Moreover, our data intriguingly suggest the targeted contribution of lncRNAs in a very precise step of this stress response, the negative regulation of translation. This work therefore provides invaluable novel knowledge to 1) further understand the role of lncRNAs in the DA stress response and 2) identify the critical elements of the stress response that are altered in PD and specifically trigger DA neuronal degeneration.

Mots-Clés: mitochondria, stress, dopaminergic, degeneration, stress response, long non coding RNA, lncRNA, non coding elements, LUHMES, PERK, UPR, ATF4, CHOP, XBP1, ATF3, ISR, negative regulation of translation, EIF4F, mTOR